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THE OCCURRENCE AND DISTRIBUTION OF METHANE
IN THE MARINE ENVIRONMENT

by

LARRY PHILIP ATKINSON

A thesis submitted in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE

UNIVERSITY OF WASHINGTON

1966

Approved by _____

Department _____

Date _____

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THE OCCURRENCE AND DISTRIBUTION OF METHANE
IN THE MARINE ENVIRONMENT

ABSTRACT

The distribution of methane in several anoxic marine environments is described. Maximum concentrations observed were 70 μ mole CH_4 -C/liter. The distribution of methane seems to follow that of sulfide. The data indicate that methane is derived from organic compounds not containing nitrogen or phosphorus and that its formation is much slower than that of sulfide.

INTRODUCTION

Objective

The objectives of this work are to describe the distribution and discuss the bacteriological and spatial sources of methane in several anoxic marine environments.

Previous Work

Strøm (1957), in the first published report on the detection of methane in a marine environment, observed "enormous quantities of methane" in the saline, anoxic bottom water of Rørholtfjorden, a stranded fjord in southern Norway. He (Strøm 1961) also observed methane in the saline, anoxic bottom water of Botnvatn, a stranded fjord in the Salten district of northern Norway. Holtan (1965) observed methane in the saline, anoxic bottom waters of Tronstadvatn and Birkelandvatn, two stranded fjords in the Kristiansand area of southwestern Norway. Williams, Mathews, and Pickard (1961) detected methane in the anoxic deep water of Powell Lake, a stranded fjord on the southern coast of British Columbia. Kriss (1949) stated that methane is present in the depths of the Black Sea, however, he (Kriss 1962) later stated that methane is probably present in large amounts but he presents no data or references to support such a claim. Karababa (1964) observed no gas bubbles in samples taken from depth in the Black Sea and concluded that no methane was present. Emery and Hoggan (1958), using mass spectrometric analysis of extracted gas, reported the presence of

methane in the sediment of the Santa Barbara Basin. They stated methane was not present in the water column, but presented no substantiating data. Revelle (1950) stated, "appreciable amounts of an inflammable gas, possibly methane, were present in the lower parts of several cores of diatomaceous mud" in the Gulf of California, but made no mention of methane in the water column.

Location and Description of Areas Investigated

Lake Nitinat, an anoxic fjord, is located on the southwestern coast of Vancouver Island, British Columbia at 48°50' N lat, 124°25' W long, (Northcote, Wilson, and Hurn 1964). The fjord is 23 km long and averages 1.2 km wide (Fig. 1). The entrance is 2.7 km long, about 4 m deep at high tide, and averages 40 m in width. Several streams enter the fjord near the head. The restricted entrance and freshwater influx result in a strong halocline that restricts vertical circulation resulting in anoxic conditions below about 30 m. The concentration of hydrogen sulfide increases with depth, reaching concentrations greater than 300 $\mu\text{g-at. S}^{2-}$ /liter near the bottom (Richards et al. 1965).

The Cariaco Trench (Richards and Vaccaro 1956) is a 1,400 m-deep depression in the continental shelf off the Venezuelan coast at 11°40' N lat and 65° W long (Fig. 2). The deepest sill is at about 150 m, effectively restricting the circulation of deeper Caribbean water. Water in the trench below 200 m is virtually isothermal and isohaline at 16.9C and 36.2‰, respectively. Because of the restricted deep-water circulation and a permanent thermocline, the waters below about 400 m are devoid of oxygen. Sulfide concentrations reach 20 $\mu\text{g-at. S}^{2-}$ /liter at the bottom.

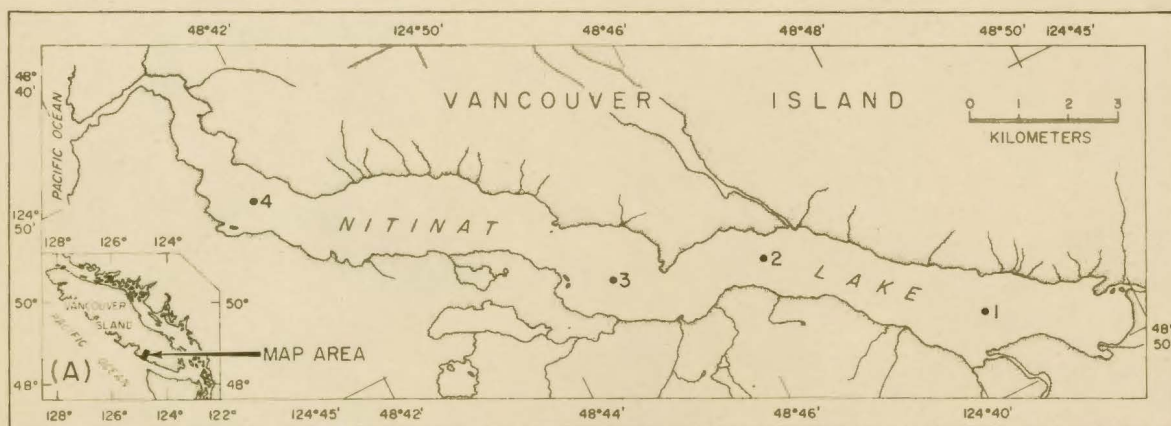


Fig. 1a. Station plan, Lake Nitinat June 1965 and May 1966.

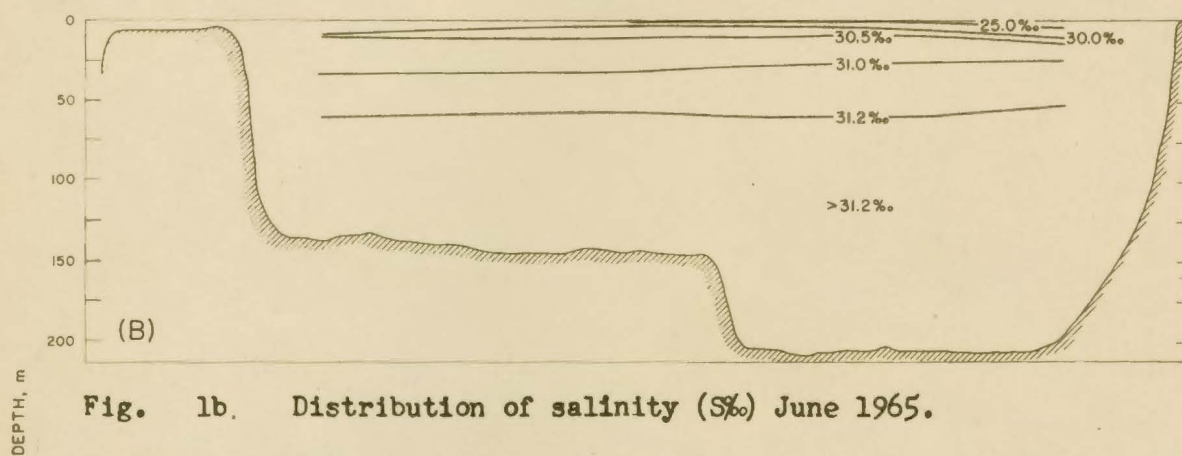


Fig. 1b. Distribution of salinity (‰) June 1965.

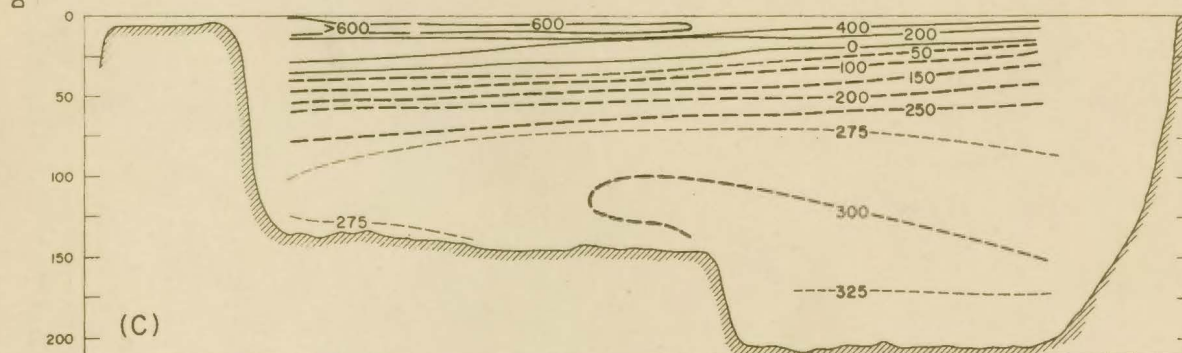


Fig. 1c. Distribution of oxygen (μg-at./liter) — and hydrogen sulfide (μg-at./liter) --- June 1965.

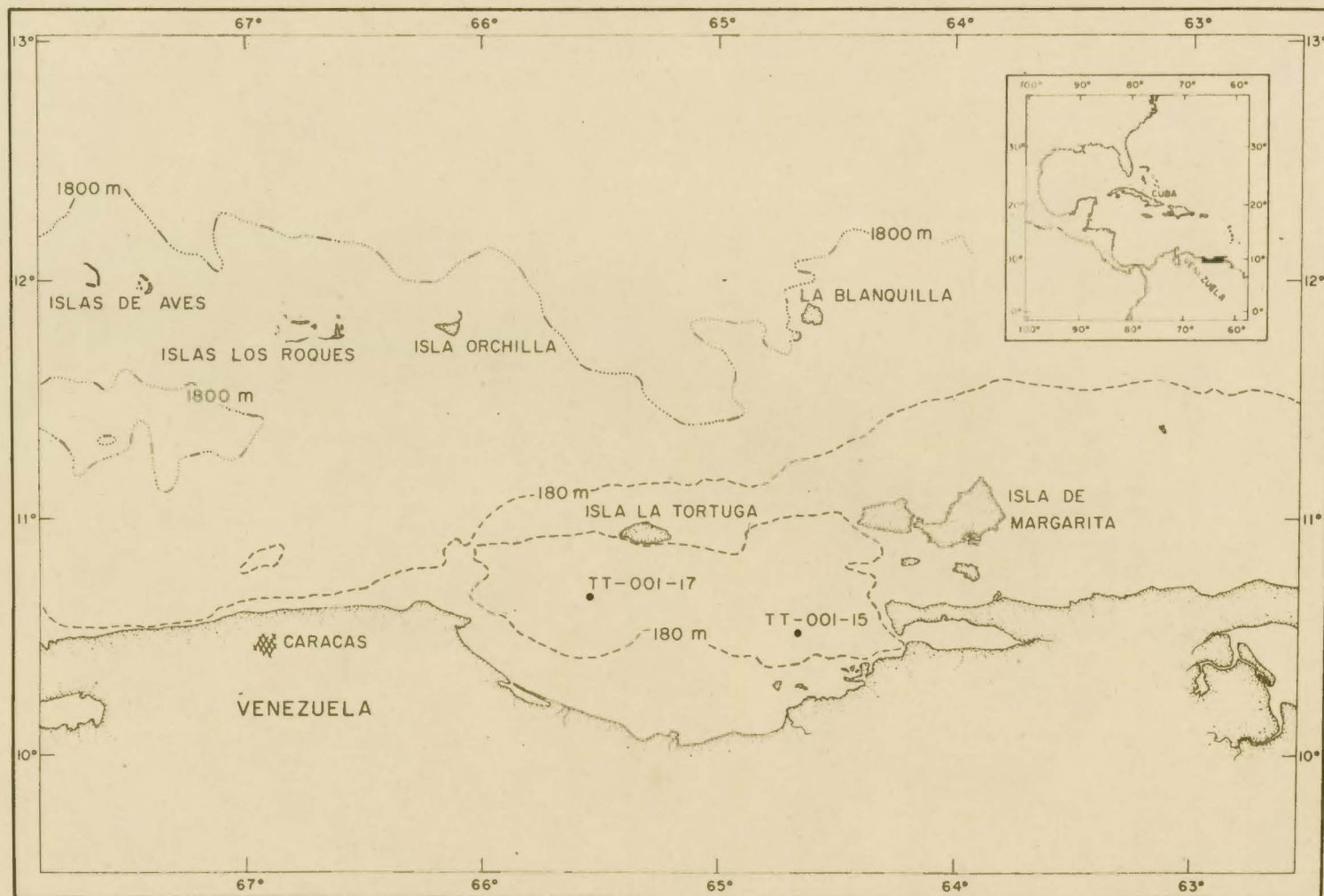


Fig. 2 Station plan and bathymetry, Cariaco Trench November 1965.

The Black Sea (Caspers 1957) is separated from the Mediterranean Sea by the Bosphorus that restricts the inflow of relatively dense Mediterranean water. This, combined with an excess of precipitation and runoff over evaporation, results in a strong vertical stability leading to anoxic conditions below 125 to 225 m. Sulfide concentrations reach 360 $\mu\text{g-at. S}^{2-}/\text{liter}$ near the bottom.

Saanich Inlet, British Columbia (Herlinveaux 1962) is a fjord on the southeastern side of Vancouver Island at 48°40' N lat and 123°30' W long (Fig. 3). It is 26 km long and about 3 km wide, narrowing towards the head. The maximum depth is 234 m and the sill depth is 70 m. Anoxic conditions develop seasonally below about 150 m. Sulfide concentrations reach a maximum of about 30 $\mu\text{g-at. S}^{2-}/\text{liter}$ near the bottom.

The Santa Barbara Basin is a 570-m deep depression in the continental shelf off Santa Barbara, California at 34°10' N lat and 120° W long. The sediment (Emery and Hoggan 1958) and the overlying 5 m of water are anoxic (unpublished data of the author).

The eastern tropical Pacific Ocean has a large area in which the oxygen concentration nearly reaches zero. This area extends west from the Mexican coast between the Equator and 25° N lat (Bennett 1963).

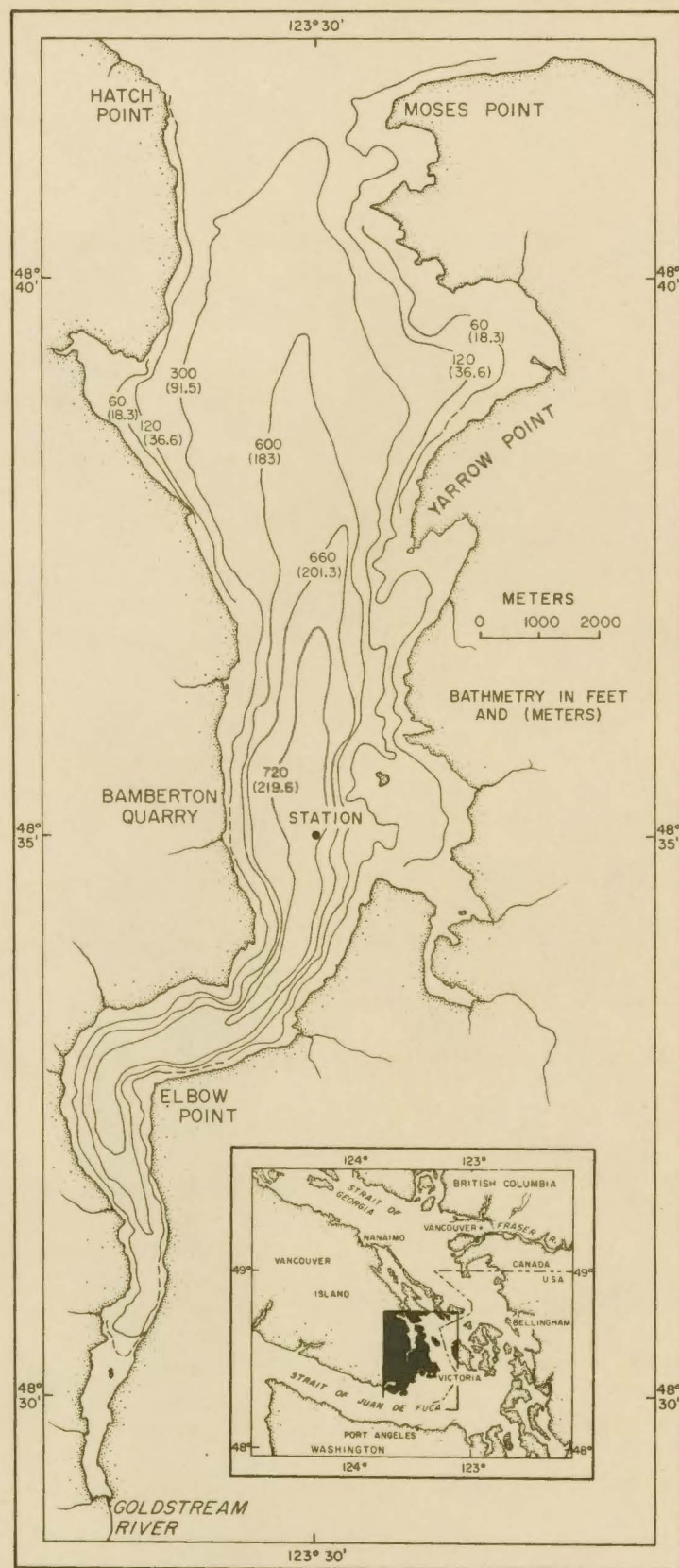


Fig. 3 Station plan and bathymetry, Saanich Inlet June 1966.

SAMPLING AND LABORATORY METHODS

Field Work

Table 1 summarizes the field work. All samples were taken in non-metallic water sampling bottles built from a modification of the Emsworth design of Carruthers, Stubbings and Lawford (1950).

During the Cariaco Trench (TT-001-15 & 17), eastern tropical Pacific Ocean (TT-001-58), Santa Barbara Basin (TT-001-73), June 1965 Nitinat (HH-196), May 1966 Nitinat, and the June 1966 Saanich investigations, a modification of the gas stripping technique of Swinnerton, Linnenbom, and Cheek (1962) was used (see Appendix II for details of the method). Samples on TT-001 and HH-196 were drawn in citrate bottles normally used for salinity samples. Samples were drawn in a manner similar to that used for the Winkler oxygen analysis and stored at 1C. The lower temperature increases the solubility of methane and therefore slows the loss of it from solution. During TT-001 and HH-196, samples were also drawn for analysis at the laboratory. Gas ampoules (250 ml) were slowly filled to overflowing, about 1 ml of mercuric chloride slurry was injected to stop bacterial action, the stopcocks were closed securely, and the ampoules were stored in a cool, dark place. During the May 1966 Nitinat and June 1966 Saanich Inlet investigations, water samples were drawn in 6-ounce square bottles and 50-ml serum bottles and about 0.5 ml of mercuric chloride solution was injected to stop bacterial action. They were sealed with serum caps and returned to the laboratory for analysis.

TABLE 1

Summary of field work

<u>General Location</u>	<u>Date</u>	<u>Vessel</u>	<u>Station</u>	<u>Location</u>	<u>Method of Analysis</u> ⁴
Black Sea	29-IX-64	CANLILARI CANLILARI	AGS-2-009 ¹	41°58'N, 30°23'E	GE
Lake Nitinat	20-VI-65	R.V. HOH	HH-196-3	48°45'N, 124°45'W	GE and GS
Cariaco Trench	2-XI-65	R.V. THOMPSON	TT-001-15	10°31'N, 64°38'W	GE and GS
Cariaco Trench	4-XI-65	R.V. THOMPSON	TT-001-17	10°40.5'N, 65°32.5'W	GS
East Tropical Pacific	29-XI-65	R.V. THOMPSON	TT-001-58	9°9.5'N, 88°59.7'W	GS
Santa Barbara Basin	11-XII-65	R.V. THOMPSON	TT-001-73	34°16.0'N, 120°42'W	GS
Lake Nitinat ²	21-V-66			48°49'N, 124°42'W	GS
Saanich Inlet ^{2, 3}	4-VI-66			48°35'N, 123°30'W	GS

¹ Samples taken by F. A. Richards in cooperation with the Turkish Navy Hydrographic Office.

² Small unnamed boats used.

³ Samples taken in cooperation with Pacific Oceanographic Group of the Fisheries Research Board of Canada.

⁴ GE = gas extraction analysis, GS = gas stripper analysis.

Gas Chromatographic Analysis

Gas-solid chromatography (Keulemans 1959) involves the transport of a mixture of gases through a stationary phase by a moving phase. The stationary phase selectively retards the gas being transported, and the components of the gas are separated and eluted from the column at different times. As the separated components leave the column, they pass a hot-wire filament that gives a measure of the thermal conductivity of the gas mixture passing it relative to the thermal conductivity of the pure moving phase.

The Model 29 Fisher-Hamilton Gas Partitioner was used and the modifications necessary are described in Appendix I. The stationary phase was Molecular Sieve (5A) vacuum packed in a 6' x 1/4" column, and the moving phase was dry helium. The signal from the detector was recorded with a Texas Instruments 1-millivolt recorder.

The gas stripping technique (Swinerton, Linnenbom, and Cheek 1962) involves the quantitative injection of the water to be analyzed into a glass chamber through which the carrier gas (helium) is flowing. Methane and other gases are stripped from the sample by the helium and carried to the partitioner where the amount of methane is determined. A detailed description of this method is given in Appendix II.

The gas extraction technique involves the vacuum extraction of gas from a known volume of seawater with a high vacuum apparatus. The volume of the gas is then measured manometrically (Broenkow 1963). The extracted gas is transferred with a gas pipette to the gas partitioner where the gas is removed quantitatively from the pipette and injected into the partitioner with a gas-tight syringe (see Appendix III for details).

RESULTS

The results of the June 1965 investigations at station 3 in Lake Nitinat are shown in Fig. 4. The discrepancy between the values obtained by the gas stripping technique and those obtained by gas chromatographic analysis of the extracted gas may have arisen because the samples to be extracted were stored for a longer time, and leakage may have occurred. The methane maximum between 90 m and the bottom as shown in the gas stripper data profile is not significant and it is doubtful if the indicated negative gradient at the bottom is significant. The methane profile derived from the analysis of extracted gas indicates a slight increase at the bottom which is not significant. This profile also indicates methane at 30 m, where the gas stripping data indicated none was present. The samples for gas extraction were gathered eight days after the gas stripping analysis and at a slightly different location, and this could account for the different 30-m values.

The results of the May 1966 Lake Nitinat investigations at station 1 are shown in Fig. 5. The sulfide and oxygen data are from the June 1965 cruise and although the conditions in the surface layers may not have been the same, the conditions at depth probably were. Fig. 6 shows the gas stripping data of 1965 at station 3 and the gas stripping data of 1966 at station 1. The significance of the profile is the apparent constant value of about 55 μ mole CH_4 -C/liter.

The results of the Cariaco Trench investigations are shown in Figs. 7 and 8. Station 15 shows a steady increase of methane to the bottom with

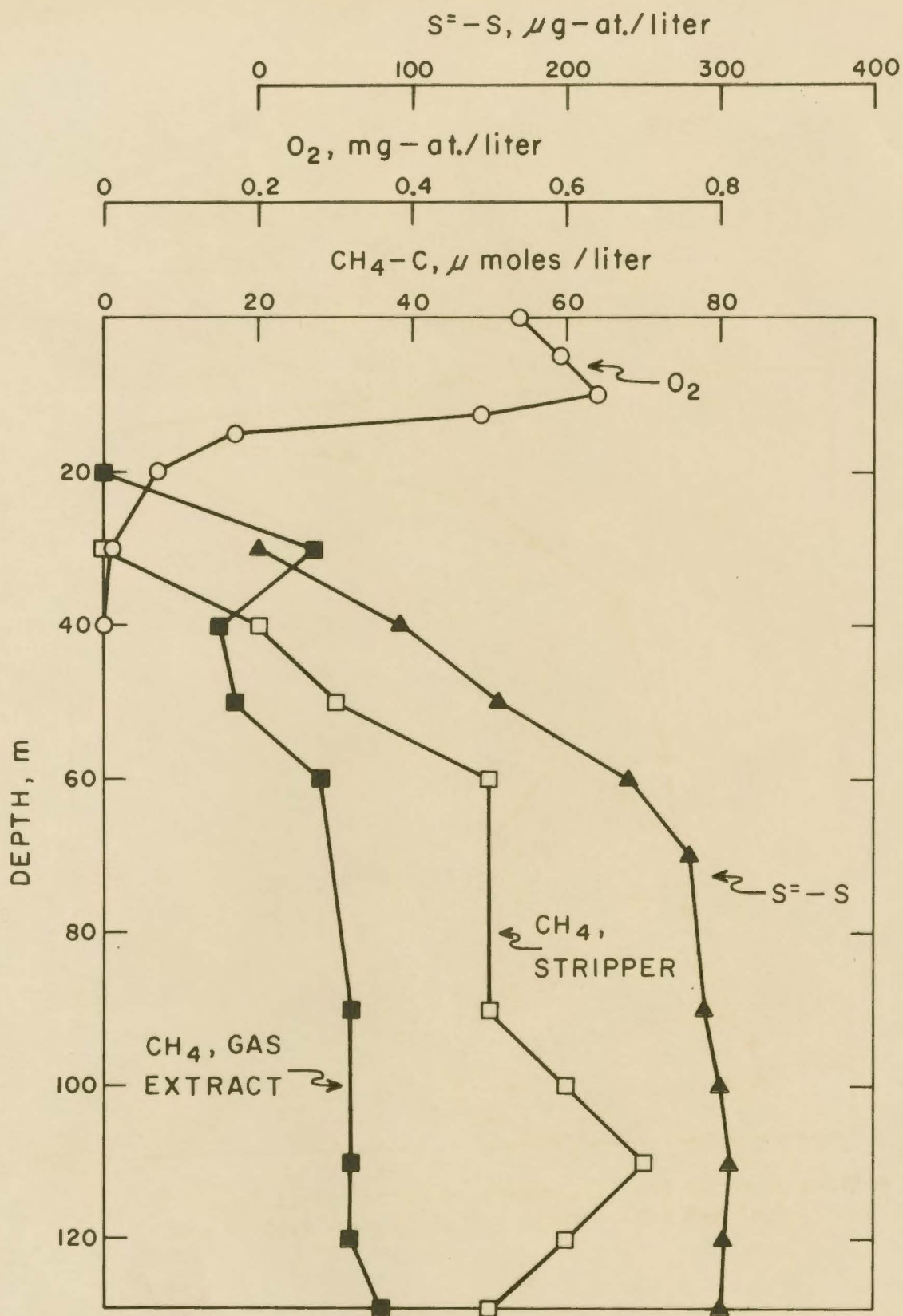


Fig. 4 Vertical distribution of oxygen (O), hydrogen sulfide (\blacktriangle), and methane (\blacksquare & \square), Lake Nitinat June 1965.

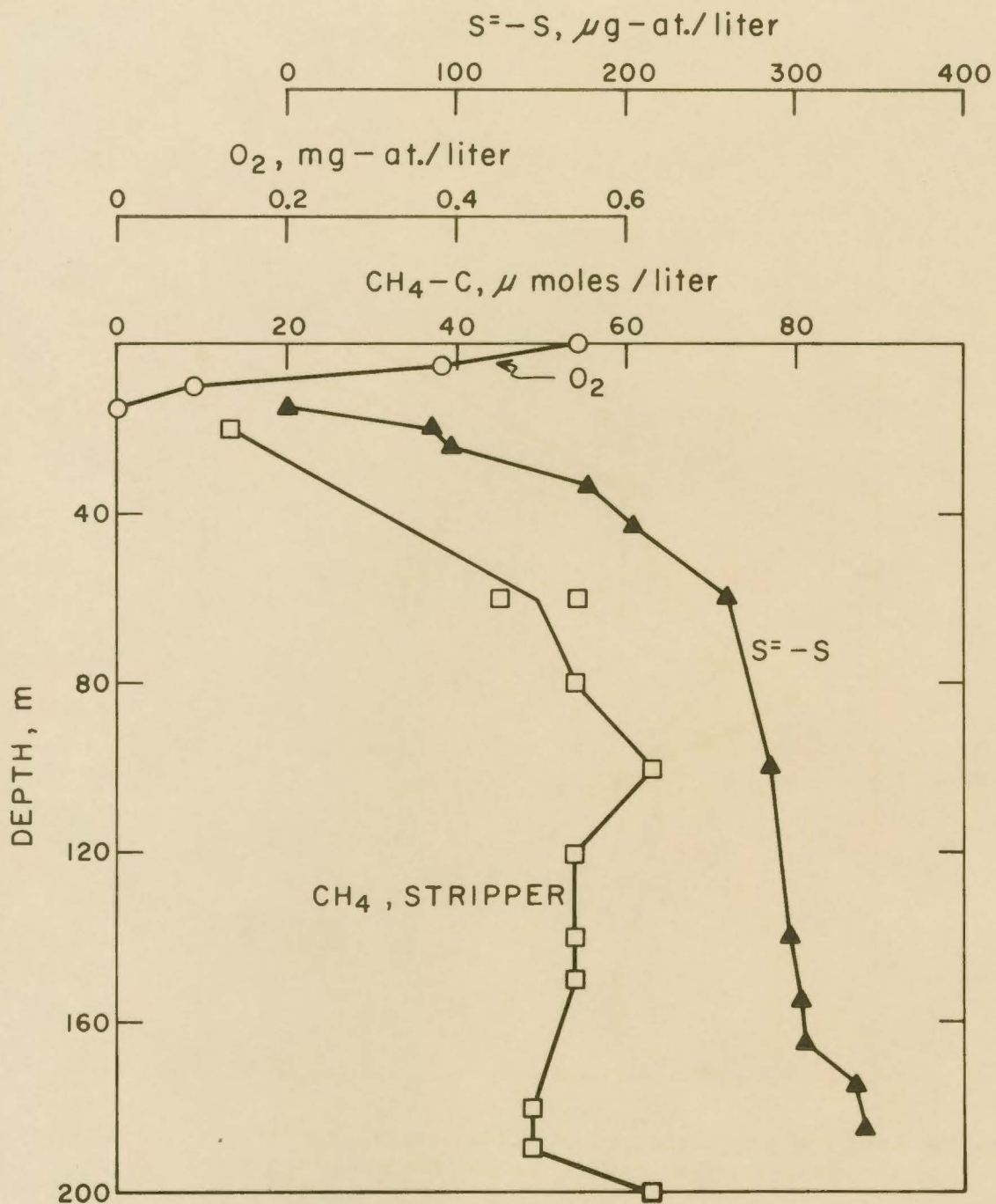


Fig. 5 Vertical distribution of oxygen (O) and hydrogen sulfide (Δ), Lake Nitinat June 1965, and methane (\square) May 1966.

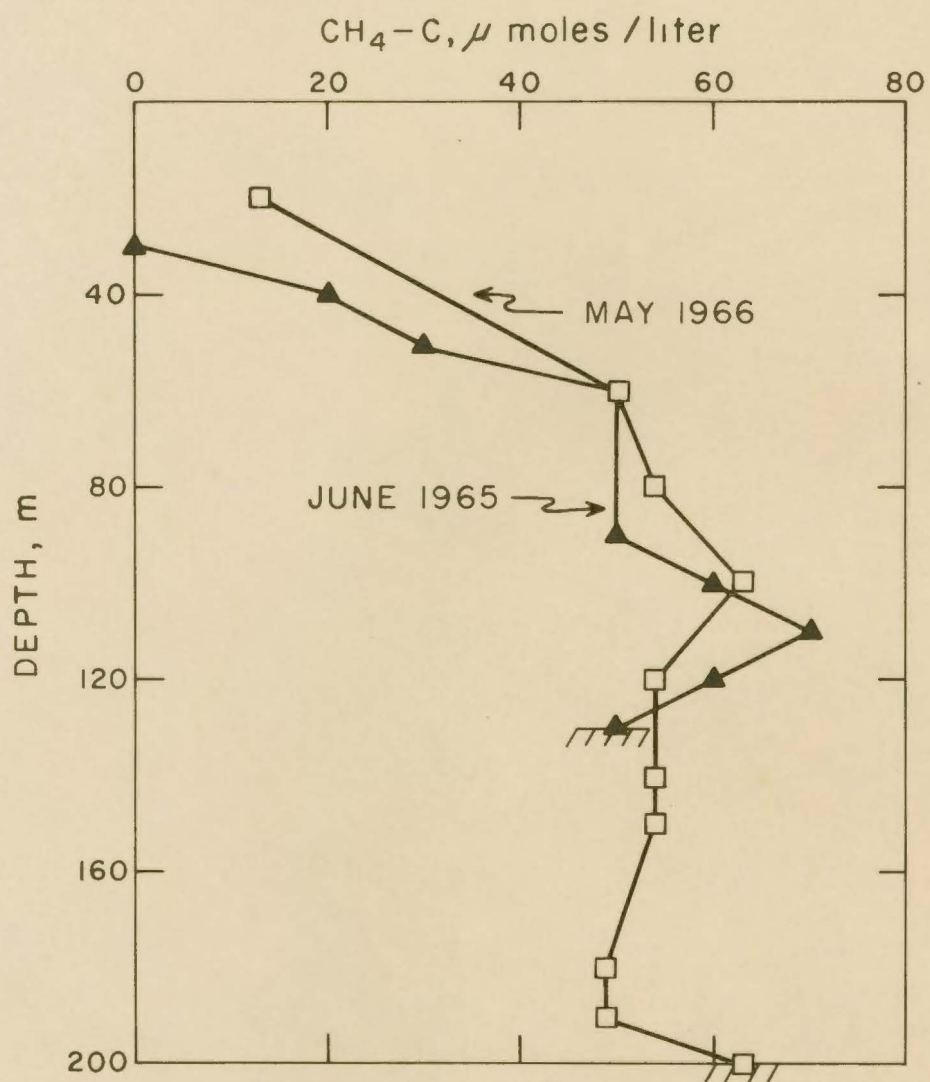


Fig. 6 Vertical distribution of gas stripping analyzed methane (\blacktriangle), Lake Nitinat June 1965, and (\square) May 1966.

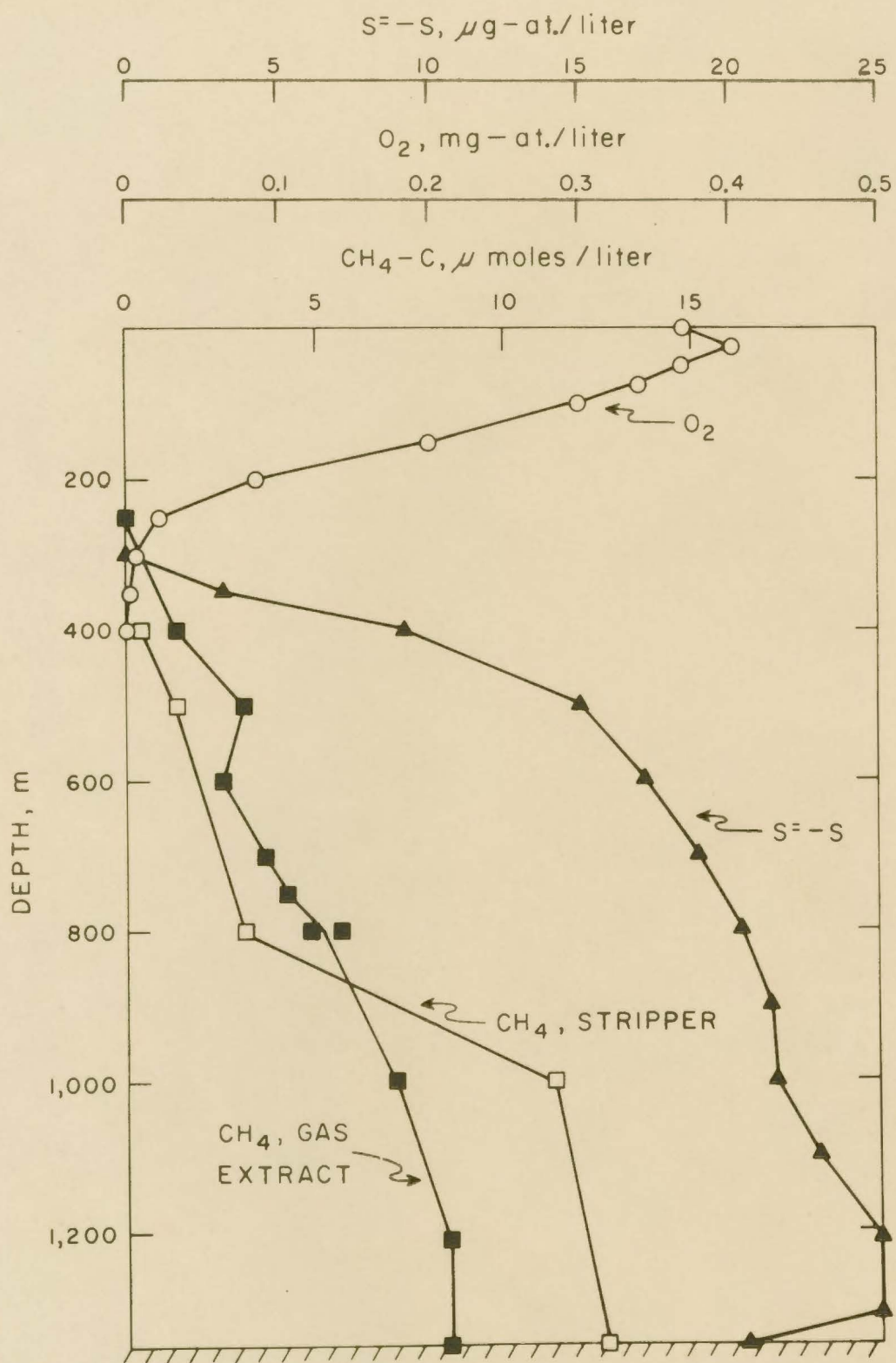


Fig. 7 Vertical distribution of oxygen (O), hydrogen sulfide (Δ), and methane (\square & \blacksquare), at station TT-001-15, Cariaco Trench November 1965.

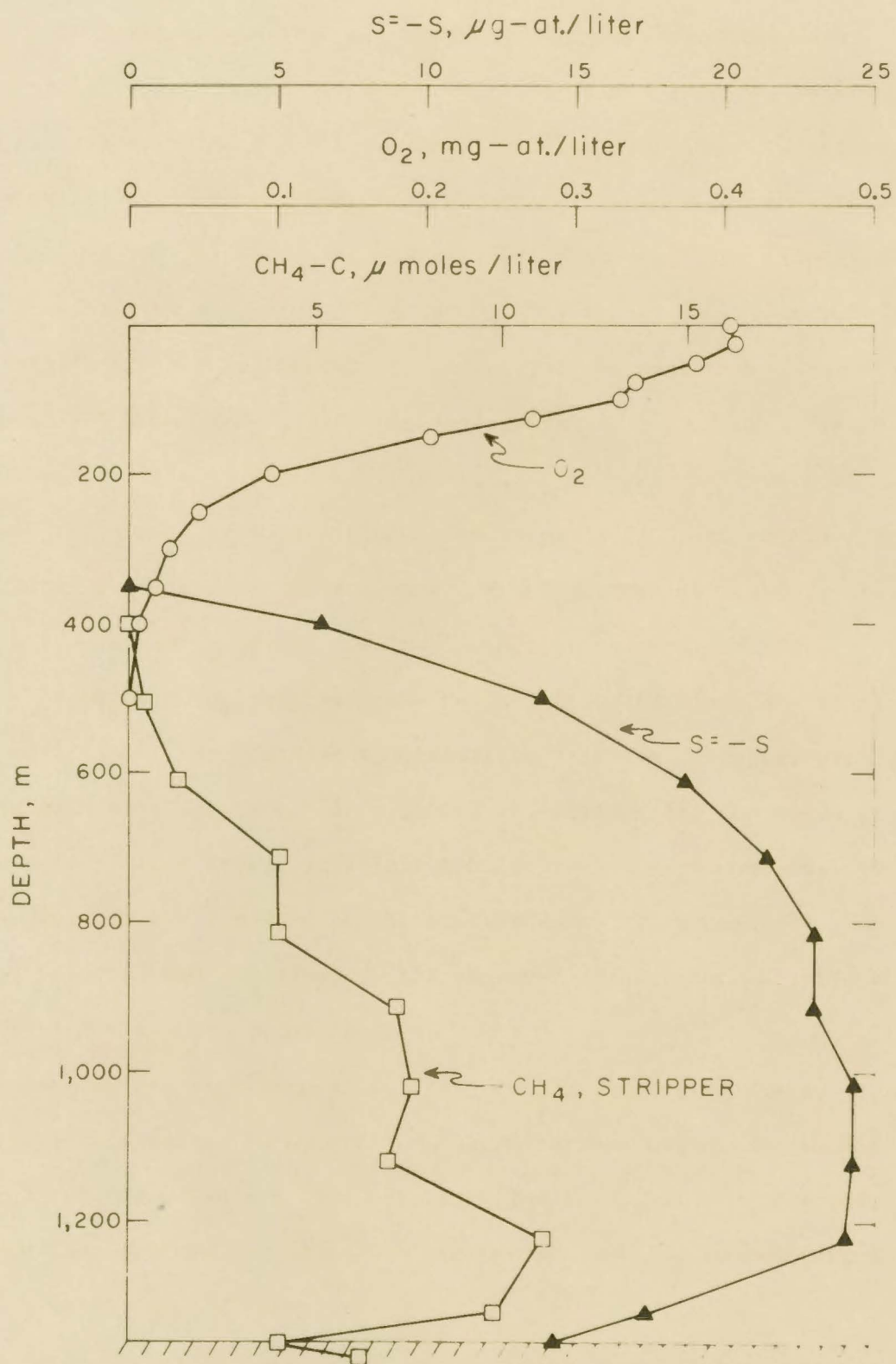


Fig. 8 Vertical distribution of oxygen (O), hydrogen sulfide (\blacktriangle), and methane (\square), at station TT-001-17, Cariaco Trench November 1965.

fairly good agreement between gas stripper and gas extraction data. The divergence of values below 900 m cannot be explained readily, especially since the values above 900 m do agree. The gas stripping data were obtained at sea while working with very small amounts of methane and therefore are probably less reliable than the gas extraction values. The precision of the gas extraction method at 800 m was 5.2 ± 0.4 μ mole CH_4 -C/liter. Station 17 is generally similar to station 15, except for the marked drop in methane in the lower 100 m which coincides with the decrease in sulfide. Fig. 9 shows the methane profile at stations 15 and 17. The large difference at 1,000 m probably represents loss during sampling or analysis. The negative gradient at station 17 corresponds to the negative sulfide gradient and is probably associated with an influx of deep water through the western sill.

The methane, oxygen, and sulfide data from the Black Sea station are shown in Fig. 10. The methane concentration increases steadily with depth to a maximum of about 10 μ mole CH_4 -C/liter. Except for the value at 500 m, the methane profile nearly parallels that of the sulfide. Because these samples were stored for 9 months before analysis, it is probable that some leakage occurred and consequently the observed methane concentrations are probably less than the actual values.

Because of interferences from the ship's electrical system it would not have been possible to detect low concentrations of methane in the eastern tropical Pacific Ocean had they existed. The examination of chromatograms suggests that methane may have been present in very low concentrations (less than 0.1 μ mole CH_4 -C/liter).

In the Santa Barbara Basin, as in the eastern tropical Pacific Ocean,

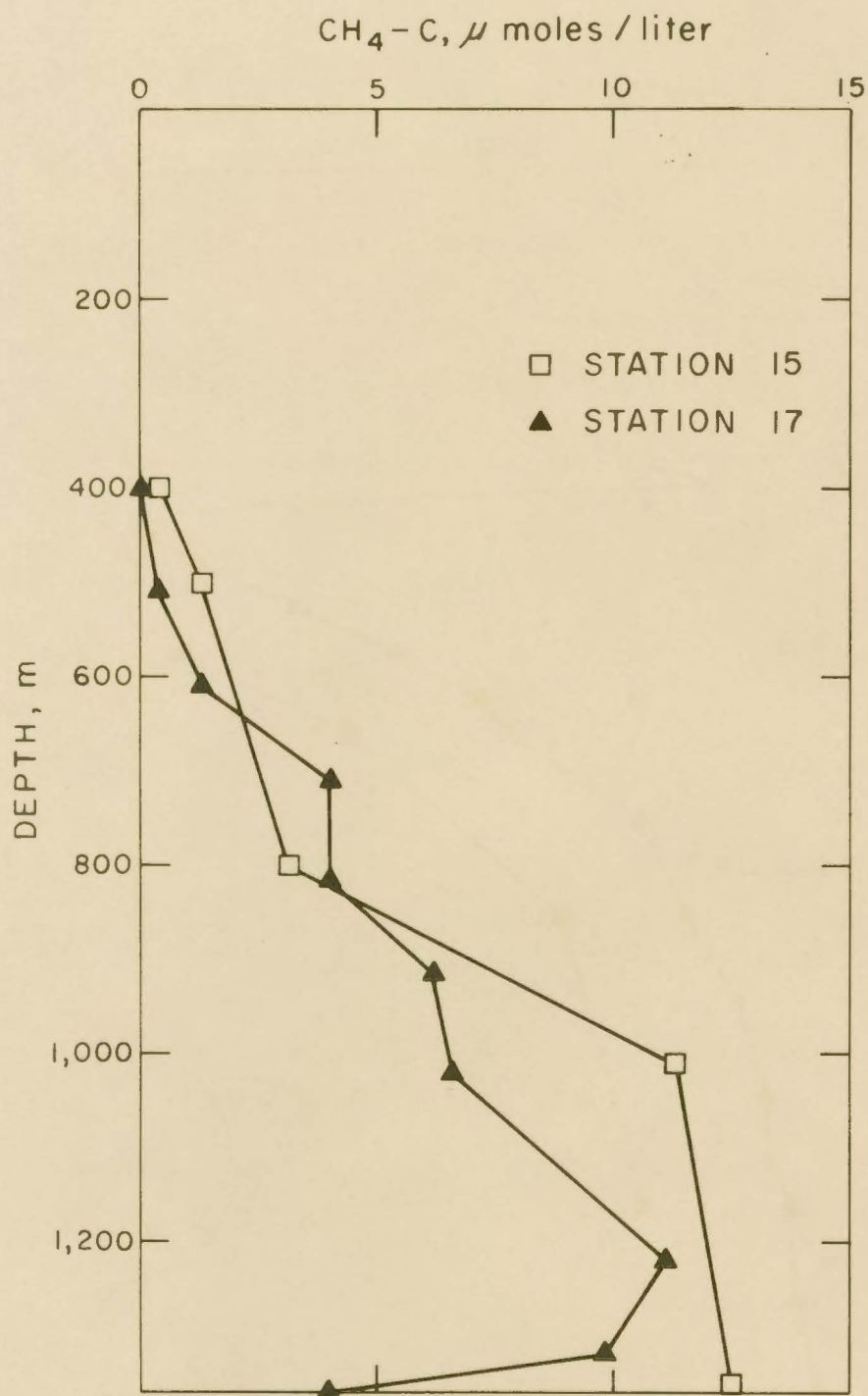


Fig. 9 Vertical distribution of methane at stations TT-001-15 (□) and TT-001-17 (▲), Cariaco Trench November 1965.

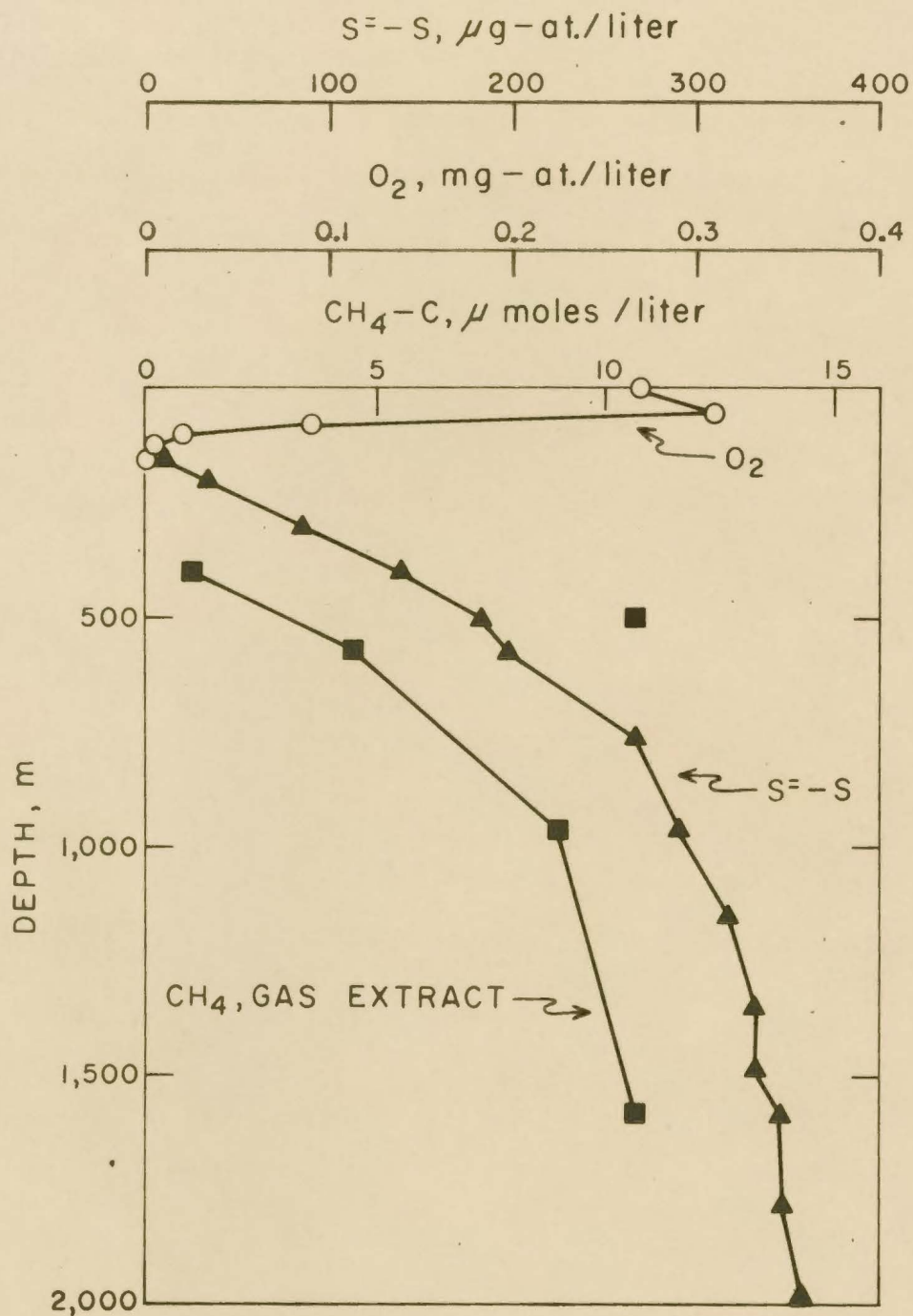


Fig. 10 Vertical distribution of oxygen (O), hydrogen sulfide (\blacktriangle), and methane (\blacksquare), Black Sea September 1964.

the electrical interference rendered the chromatograms useless for the detection of low concentrations of methane.

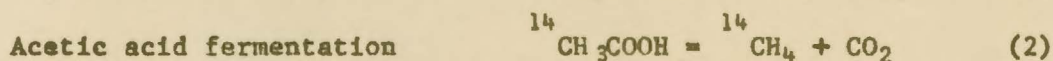
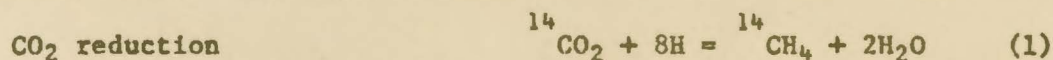
In Saanich Inlet, methane concentrations of 0.01 to 0.04 μ mole $\text{CH}_4\text{-C/liter}$ were observed in the 230-m sample that contained mud. Sulfide was found in the lower 70 m in concentrations increasing from zero at 150 m to 27 $\mu\text{g-at. S}^{\text{--}}\text{-S/liter}$ at 220 m.

DISCUSSION

Sources of Methane

The methane found in anaerobic environments can arise from either the bacterial decomposition of organic matter in the water column (or sediment), or seepage from surrounding rock which may contain methane. Oil seeps are known to occur along the southern California coast (Emery 1960) and presumably methane is released with the oil, but it is doubtful if this is a significant source of methane in the areas investigated.

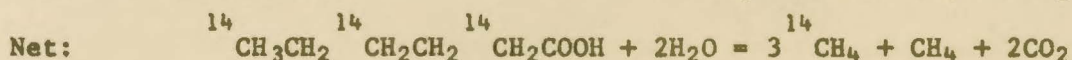
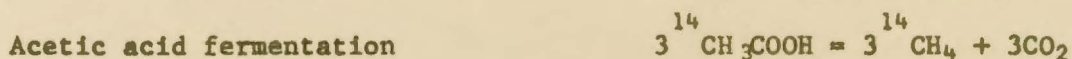
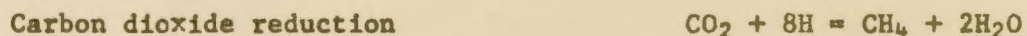
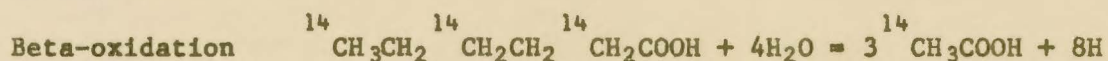
Bacteriologically, methane can be formed either from carbon dioxide reduction or acetic acid fermentation (McCarty 1964; Pine and Barker 1956; Koyama 1963) under anaerobic conditions as follows (the ^{14}C indicates the pathway of the methyl carbon):



Methanol fermentation is a third possible source of methane (Stadtman and Barker 1951), but methanol is not a common degradation product and therefore is probably a relatively minor source of methane.

The relative importance of reactions (1) and (2) can be seen by examining the results of anaerobic fermentation of various organic material. Fatty acids will be released to the water as a result of the hydrolysis of fats and oils. Short chain fatty acids result from the fermentation of carbohydrates and proteins. McCarty (1964) reports that during the fermentation of even-numbered carbon fatty acids, 75% of the methane resulted from acetic acid fermentation of the beta-oxidized remains (acetic acid groups) of the larger fatty

acid. One-fourth of the methane comes from carbon dioxide reduction by the excess hydrogens atoms resulting from the original beta-oxidations that produced the acetic acid groups. An example of this is the fermentation of caproic acid:



During the fermentation of odd-numbered fatty acids, the beta-oxidation produces acetic acid groups until there is a three-carbon propionic acid group left. This is then fermented, resulting in about 60% of the methane arising from acetic acid fermentation. The remainder arises from carbon dioxide reduction utilizing the hydrogen atoms released during the beta-oxidation and propionic acid fermentation. An example of this is the fermentation of valeric acid ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{COOH}$) in which 50% of the methane results from acetic acid fermentation and 50% from carbon dioxide reduction. The consequence of the fermentation of higher odd-numbered carbon fatty acids is that a larger fraction of the methane arises from acetic acid fermentation. Methane fermentation of propionate might be of considerable importance because propionate is a major product of the fermentation of complex organic compounds.

The fermentation of proteins follows their hydrolysis to their constituent amino acids. Either the amino acid is oxidized, decarboxylated, and converted to a saturated acid with one less carbon atom than the original amino acid, or reduction occurs and a saturated acid with the same number of carbon atoms results. In all cases, deamination takes place with the

release of ammonia. The saturated acid is then fermented as a carbohydrate. McCarty (1964) reports from studies of the fermentation of nutrient broth that 28% of the methane resulted from carbon dioxide reduction and 72% from acetic acid fermentation. The most abundant carbohydrates in nature are the five and six carbon monosaccharides and in some environments cellulose may also be one of the predominant carbohydrates. The methane fermentation of one mole of six-carbon glucose (McCarty 1964) results in three moles of methane, two from acetic acid fermentation and one from carbon dioxide reduction, or, 67% of the methane comes from acetic acid fermentation. The fermentation of one mole of pentoses will result in 2.5 moles of methane, two moles from acetic acid fermentation and one-half mole from carbon dioxide reduction. In this process, 80% of the methane results from acetic acid fermentation.

ZoBell (1939) and McCarty (1964) state that anaerobic fermentation of carbohydrates is the primary mechanism in methane production. Zobell points out that bacteria are known to ferment carbohydrates (including cellulose) anaerobically in recent marine sediments. This fermentation results in a mixture of products that includes low-carbon fatty acids, carbon dioxide, and methane. The fact that the carbohydrate concentration in recent sediments is 1% of the total organic content whereas in ancient sediment carbohydrate is absent, indicates that anaerobic fermentation of carbohydrates to methane, fatty acids, and carbon dioxide has taken place. McCarty (1964) states that more methane synthesis results from carbohydrate fermentation than from fatty acid or protein fermentation because of the additional energy available in the conversion of the carbohydrate to an

organic acid. It is evident that methane can result from many fermentative and reductive pathways with fermentative oxidation of carbohydrates being the most important.

McKinney and Conway (1957) proposed a thermodynamic model in which organic matter is oxidized by bacteria first using free oxygen as a hydrogen acceptor, followed by nitrate, then sulfate, and finally acetate. The free energy yields of the reactions in which acetate is oxidized are shown in Table 2.

TABLE 2

Free energy yield upon acetate oxidation using various reductants

<u>Energy source</u>	<u>Free energy yield (kg-cal/mole acetate)</u>
O ₂	-206.9
NO ₃ ⁻	-187.0
SO ₄ ⁻	-11.2
acetate fermentation	-7.0

Thermodynamically, the bacteria obtain more energy from oxidation at the expense of free oxygen than nitrate reduction and more energy from nitrate reduction than from sulfate reduction. Richards (1965) has shown that this thermodynamic model partially explains the results of the decomposition of organic matter in oxygen-low and oxygen-deficient environments. The thermodynamic model predicts that methane will not be formed in the presence of sulfate. However, as the data presented demonstrate, methane is present in some marine environments. There are three possible explanations of this:

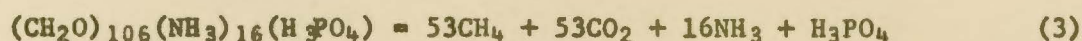
It is possible that in micro-environments, for example around organic particulate matter where accelerated sulfate reduction has occurred due to the high concentration of substrate, the sulfate concentration drops sufficiently to permit methane production.

Sulfate may be absent in the sediment as a result of sulfate reduction and restricted replenishment of sulfate-rich water from the water column. In this environment, methane production would be thermodynamically possible. The methane produced could then diffuse and be advected, via bubbles, into the water column.

One could ignore the thermodynamic model and state that if there is a mechanism by which a species of bacteria can live and reproduce, it will. It should be remembered that we are not working with a single species of bacteria that, when presented with several choices of energy sources, will pick the most advantageous. If this were the case, one would expect the thesis of McKinney and Conway to apply strictly. The sulfate reducers incompletely oxidize their organic substrate and commonly have short-chain fatty acids as by-products (Leban and Wilke 1963). The methane bacteria generally require short-chain fatty acids as substrate. If short-chain fatty acids are present as by-products of sulfate reduction, the methane bacteria also are present to utilize the favorable condition of suitable substrate and anaerobic conditions. Conversely, if the sulfate reducers did oxidize their substrate completely to carbon dioxide and water, no methane producers would be expected. Therefore, it seems possible that methane is produced during the anaerobic fermentation of the low molecular weight by-products of the sulfate reducing bacteria.

Effect of Methane Fermentation on the Ratios of CO_2 , S^{--} , NH_3 , and PO_4^{--3}
in the Water Column

Methane formation will affect the water column by either liberating or consuming carbon dioxide, ammonia, phosphate, and methane. Richards et al. (1965) propose that one representation of methane production might be:



The $\Delta\text{CO}_2:\Delta\text{CH}_4:\Delta\text{NH}_3:\Delta\text{H}_3\text{PO}_4$ ratio would be 53:53:16:1, by moles. Equation (3) represents the anaerobic fermentation of average organic matter (Richards 1965) in which methane is formed from the carbohydrate, (CH_2O) , units. This is a good representation of the reaction because, as stated previously, the primary source of methane probably is the anaerobic fermentation of carbohydrates. During sulfate reduction, $\Delta\text{CO}_2:\Delta\text{S}^{--}:\Delta\text{NH}_3:\Delta\text{H}_3\text{PO}_4 = 106:53:16:1$, by moles (Richards 1965). Methane production as proposed would alter the ratios obtained from sulfate reduction alone by an amount dependent on the relative proportion of methane and sulfide production. In Lake Nitinat, the ratio of methane to sulfide is 1:6. Therefore, taking a weighted average of the two ratios gives a corrected ratio of:

$$\Delta\text{CO}_2:\Delta\text{CH}_4:\Delta\text{S}^{--}:\Delta\text{NH}_3:\Delta\text{H}_3\text{PO}_4 = 104.2:8.0:47.7:16:1 \quad (4)$$

This mechanism for methane production does not change the ammonia-phosphate ratios. During the proposed methane formation, both carbon dioxide and ammonia would be added to the water in a ratio of $\Delta\text{CO}_2:\Delta\text{NH}_3 = 53:16$, which reduces the $\Delta\text{CO}_2:\Delta\text{NH}_3$ ratio to 104:16, lower than the 106:16 ratio expected from sulfate reduction alone and much lower than the observed ratio of 141:16.

The proposed methane formation would contribute ammonia to the system and thereby alter the $\Delta S^{\text{m}}:\Delta\text{NH}_3$ ratio to 47.7:16. In Lake Nitinat, Richards et al. (1965) observed $\Delta S^{\text{m}}:\Delta\text{NH}_3 = 50.2:16$, a ratio lower than the 53:16 expected from sulfate reduction alone and higher than the 47.7:16 predicted by Equation (4). The $\Delta\text{CO}_2:\Delta\text{NH}_3$ ratio indicates that methane formation is not contributing ammonia to the system, whereas the $\Delta S^{\text{m}}:\Delta\text{NH}_3$ data indicates it could possibly be contributing a slight amount. The ratios appear to indicate that the primary source of methane is not organic material of the type proposed, $(\text{CH}_2\text{O})_{106}(\text{NH}_3)_{16}(\text{H}_3\text{PO}_4)$, but the carbohydrate and fatty acid by-products of other processes, probably sulfate reduction. Upon fermentation, carbon dioxide, water, and methane would be released to the water column, while ammonia and phosphate might be removed from the water column.

Partial Explanation for the Low Observed Concentrations

Too little is known of the biochemical mechanisms of methane production to explain the relatively low observed concentrations. The solubility of methane in seawater (ca. 36‰) at 10°C is about 33 ml CH_4 /liter (unpublished data of the author). Therefore, it is doubtful that the low observed concentrations are a result of a saturated condition. McCarty (1964) found that the generation time of anaerobic bacteria is greater than that of aerobic bacteria. The methane producing bacteria, specifically, have a very slow generation time which averages four to five days (an average generation time for aerobic bacteria is 20 min to 3 hr) with a maximum of 15 days. McCarty also believes that low energy yields and generally low substrate utilization rates contribute to the slow generation time, and hence, the slow rate of methane accumulation.

The June 1966 data from Saanich Inlet are indicative of the slow rate of methane accumulation. Saanich Inlet probably flushes during the fall and therefore any methane or sulfide present was formed in the period following flushing. By June 1966, sulfide and methane concentrations reached 25-30 $\mu\text{g-at. S}^{\text{--}}\text{-S/liter}$ and 0.01-0.04 $\mu\text{-moles CH}_4\text{-C/liter}$. These observations indicate that in Saanich Inlet sulfide production is relatively much faster than methane production. This apparent slow rate of methane formation indicates that, following flushing, the growth of a methane producing flora is slow, possibly because some biochemical or physical parameter, such as temperature, pH, eH, or suitable substrate, is not favorable or available for growth of methane producing bacteria.

Spatial Sources of Methane

It would be possible for methane to be formed in sulfate deficient sediments and then diffuse and be advected to the water above. The data are unclear on this point. In the absence of advective replacement of bottom water, a negative gradient near the bottom would rule out diffusion from the bottom into the water column. A negative gradient near the bottom has been observed, but whether this is due to the greater production of methane in the water column than in the sediments or to the intrusion of an increment of newer bottom water is uncertain. A positive gradient near the bottom would allow diffusion processes to transport methane from the sediment into the water. Such gradients have also been observed. However, pure diffusion of methane from the sediment to the water column would result in a linear increase in the methane concentration with depth, and this has not been observed.

The transportation of methane from the sediment to the water column in bubbles is probably a real process. Echo sounder traces sometimes show what appear to be bubbles rising from the bottom of Saanich Inlet (McCartney and Bary 1965) and Lake Nitinat (unpublished data). The distribution this type of transport would produce is unknown.

The data do not indicate whether the methane is formed in the water column, the sediment, or both. More detailed sampling at the water-sediment interface might clarify this point.

Contribution to Atmospheric Methane

It is doubtful if the methane produced in the anoxic environments investigated adds substantially to the atmospheric methane. As the methane diffuses upwards out of the anoxic zone it becomes an energy source for the methane oxidizing bacteria. The only way for methane to traverse the oxygenated layer is via bubbles. In swampy and sludge-bed environments, the anaerobic zone is very near or at the surface and the methane does escape into the atmosphere.

SUMMARY

The distribution of methane in several anoxic marine environments is described. The determination of dissolved methane involves the gas chromatographic analysis of gas stripped from the water directly or extracted under a vacuum. The distribution of methane seems to follow that of sulfide. The data indicate that the methane is derived from organic compounds not containing nitrogen and phosphorus, such as carbohydrates and fatty acids. The data from Saanich Inlet suggests that methane accumulation is very slow. The uncertainty in the data does not permit the determination of the spatial source of methane.

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APPENDIX I

MODIFICATIONS TO THE FISHER-HAMILTON

MODEL 29 GAS PARTITIONER

1. Remove the drying tube assemble and bulkhead connectors. Install 1/4" stainless steel bulkhead unions (Swagelock catalog 400-61-316) in the drying tube bulkhead connector holes.
2. Mount a four-way selector valve (Republic Valve Co. catalog H-330-2) half-way between the two bulkhead unions and connect the valve and bulkhead unions with 1/8"(o.d.) heavy-walled polyethylene tubing.
3. Construct a bypass column using heavy-walled 1/4"(o.d.) polyethylene tubing and 1/4" column adapters. Install the bypass column in place of column I.
4. Column II is 6' x 1/4" and packed with Molecular Sieve (5A) preconditioned by heating at 250C for 2-4 hr.

APPENDIX II

THE GAS STRIPPING TECHNIQUE

1. Construct an all-glass sample chamber as described by Swinnerton, Linnenbom, and Cheek (1962).
2. Connect the stripper to the 4-way selector valve so that gas coming from the partitioner flows up through the glass frit and out the top of the stripper and back through the selector valve to the partitioner.
3. Adjust the gas flow to 50 ml/min with the stripper in the carrier gas line.
4. Flush the stripper for about 30 min and during this time zero the recorder.
5. Turn the cell power ON and zero the recorder with the balance controls of the partitioner.
6. Carefully draw samples to be analyzed into a gas-tight syringe and inject the water into the sample chamber at a rate such that neither does water pass down through the frit nor does gas bubble up through the water.
7. At a flow rate of 50 ml/min, the methane peak should appear in about 9.5 min. Have the partitioner on attenuation 1 and the recorder zeroed before the peak appears.
8. If an integrator is on the recorder it will automatically indicate the relative area of the peak. If there is no integrator, the area must be determined using a mechanical planimeter.

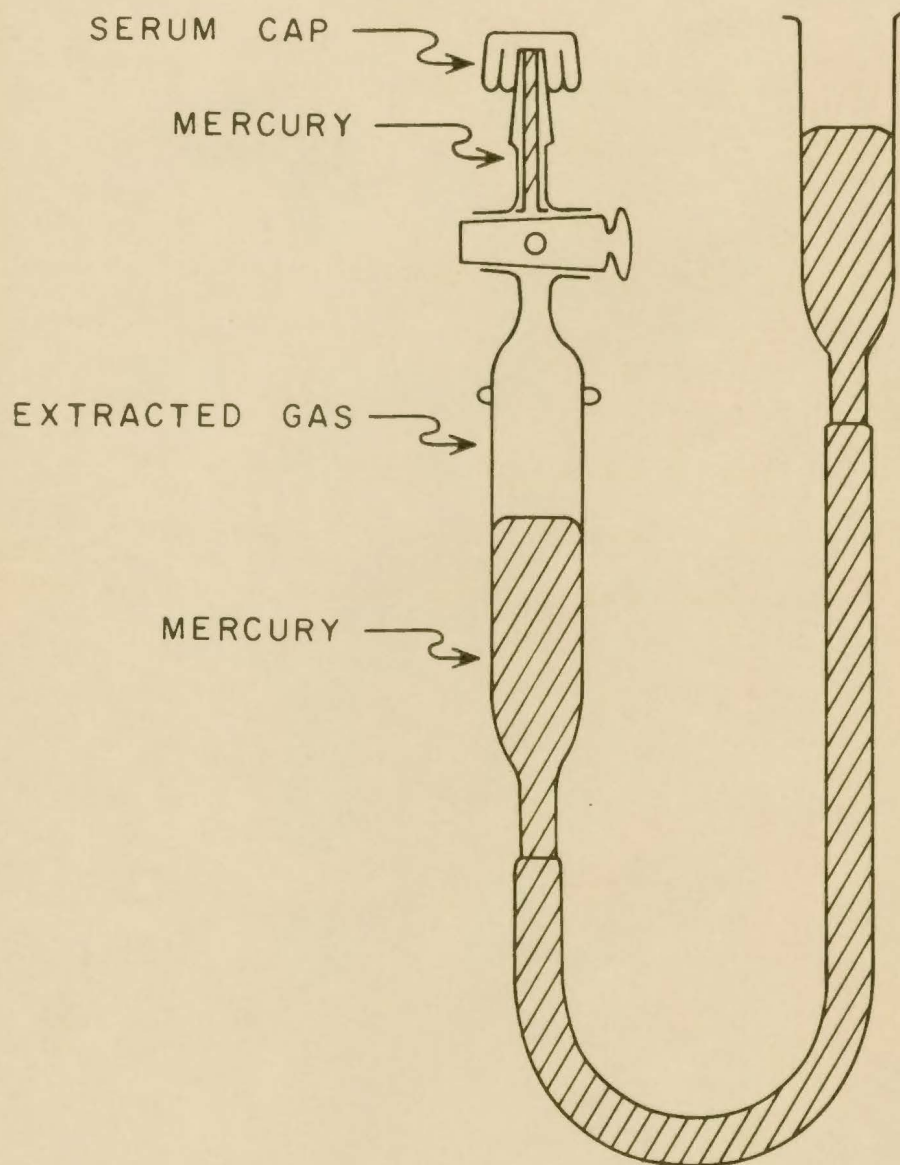
STANDARDIZATION

1. Obtain from the Matheson Gas Company a tank of gas containing 1% methane in helium.
2. Fit a short piece of polyethylene tubing to the nipple of the regulator and place a serum cap on the end of the tubing.
3. Using a 1-ml gas-tight syringe, or 5-ml if necessary to get appropriate sized peaks, quantitatively take samples from the tank and inject them into the partitioner. The area obtained from these standards then is used to determine the amount of methane represented by the areas obtained from the unknown samples.

APPENDIX III

GAS PIPETTE SAMPLING

1. Arrange the gas pipette such that there is a positive pressure on the gas contained in it (Fig. 11).
2. With a syringe fill the bore of the 10/30 male joint with mercury.
3. Fit a serum cap over the joint.
4. Open the stopcock and remove the mercury with a syringe.
5. Remove the gas to be analyzed with a gas-tight syringe: a 5 ml sample is normally the best volume if that much gas is available.
6. Inject the sample into the chromatograph and follow the directions in Appendix II from step 7 on.



GAS PIPETTE

Fig. 11 Detail of gas pipette.

APPENDIX IV

STORAGE OF WATER SAMPLES AND RECOVERY
FROM 6 OZ. SQUARE BOTTLES AND SERUM BOTTLES

1. Carefully fill the bottle to overflowing with the water to be analyzed, inject about 0.2 ml of mercuric chloride solution, and insert a serum cap securely.
2. Store the bottles in a cold, dark place during transit and storage.
DO NOT FREEZE.
3. When ready to sample, put a hole in the center of the cap with a hypodermic canulla and then push the canulla through the cap beside the first hole. Take a water sample through the first hole with a gas-tight 10 ml syringe and proceed according to Appendix II.